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Frequent Isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from Healthy Zairese Freshwater Fish: a Possible Source of Sporadic Diarrhea in the Tropics

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The intestinal contents of 59 Zairese freshwater fish were examined for the presence of potential human enteric pathogens. *Edwardsiella tarda* and *Plesiomonas shigelloides* were isolated from 57 and 59% of them, respectively. For both microorganisms there was a significant difference between the isolation rates from lake and river fish: whereas *E. tarda* was much more frequently isolated from lake fish than was *P. shigelloides*, the reverse was observed for river fish. The authors hypothesize that sporadic cases of tropical diarrhea with *E. tarda* or *P. shigelloides* can be traced to contact with or consumption of freshwater fish.

Sporadic cases of human diarrheal disease associated with *Edwardsiella tarda* or *Plesiomonas shigelloides* have previously been reported from Zaïre by Makulu et al. (15) and by Vandepitte et al. (25). The same organisms were also sporadically cultivated from different species of mammals and birds in Zaïre (L. R. Van Damme, M. N. Tshidingi, and J. Vandepitte, manuscript in preparation). During the latter investigation, *E. tarda* was also isolated from water.

During a World Health Organization mission in Mopti (Mali) in 1971, one of the authors (J. Vandepitte, unpublished observations) was impressed by the unusually high isolation rates of both *E. tarda* and *P. shigelloides* from patients with diarrhea. Since the population of Mopti is chiefly composed of fishermen and river fish are the principal source of protein food, it was hypothesized that fish could be the reservoir for human infection with these potential enteric pathogens. Further support was lent to this assumption by the isolation of *P. shigelloides* from the gills of one of five sick fish during routine examination in Kinshasa in 1977 (L. R. Van Damme, unpublished observation).

In the light of these findings, it was considered worthwhile to investigate different species of freshly caught Zaïrese river and lake fish for the presence of *E. tarda* and *P. shigelloides*.

MATERIALS AND METHODS

A total of 59 freshly caught fish were investigated from March to August 1978 (on 10 different days). Fish were caught in the following rivers or lakes: Bombo river (11 fish), Zaïre stream (23), Mfidi river (6), and four different lakes (19) (Table 1).

As soon as possible after arrival of the fish at the laboratory, samples of intestine were aseptically removed and inoculated onto different bacteriological media: (i) 28 were inoculated directly onto MacConkey agar (Eiken, Wellcome Research Laboratories); (ii) 24 were inoculated directly onto xylose lysine desoxycholate (XLD) agar (Eiken, Wellcome); (iii) 19 were inoculated onto salmonella shigella (SS) agar (Eiken, Wellcome) after enrichment for 48 h at 42°C in tetrathionate broth base (TB) (Oxoid Ltd.) with iodine solution; and (iv) 59 were inoculated onto SS agar after enrichment for 48 h at 37°C in TB without iodine solution.

All solid plating media were incubated at 37°C for 48 h. Representative types of non-lactose-fermenting colonies were picked from the solid isolation media and inoculated onto Kligler iron agar slants. Cultures giving reactions suggestive of enteric pathogens were further identified by biochemical reactions using procedures outlined by Edwards and Ewing (5). We examined some isolates in parallel, using the API 20E system for the identification of *Enterobacteriaceae*.

Antibiotic sensitivity was tested by standard disk diffusion test (1). The *Plesiomonas* strains were tested for agglutination with anti-*Shigella sonnei* serum (Wellcome).

RESULTS

A total of 35 isolates of *E. tarda* were obtained from 34 fish (57% of the fish), and 35 isolates of *P. shigelloides* were obtained from 35 fish (59% of the fish) (Table 1). One fish contained two biotypes of *E. tarda*.

The isolation rates on different media are summarized in Table 2. Intestinal samples of four different fish (catch 11, Table 1) were inoculated in parallel on the following media: XLD agar at 27, 37, and 42°C; SS agar at 37°C after enrichment in TB without iodine at 27, 37, and 42°C; TB with iodine at 37°C; and selenite broth (Difco Laboratories) at 37 and 42°C. Enrichment in selenite broth was unproductive. For

TABLE 1. *Sources, names, approximate length, and culture results of examined fish*

No. of catch	River or lake	Date of capture (1978)	No. of fish examined	Name of fish examined	Approx. length (cm)	No. positive for:	
						E. tarda	*P. shigelloides*
1	Bombo river, 120 km east of Kinshasa	3/27	1	Red tail[a]	20	4	4
			3	Sardines[a]	≥15		
2	Zaïre stream, near Kinshasa	4/3	4	*Ophiocephalus*	≤10	0	6
			3	*Clarias*	≤10		
			1	*Polypterus*	10		
3	Bombo river, 120 km east of Kinshasa	4/10	1	Red tail[a]	25	4[b]	5
			4	Sardines[a]	>20		
			2	Sardines[a]	<15		
4	Mbeo lake, about 500 km east of Kinshasa	4/27	5	*Tilapia nilotica*	≥20	5	1
5	Idiofa lake, about 500 km east of Kinshasa	4/29	5	*Tilapia nilotica*	≥20	5	1
6	Kimuenza lake, 40 km from Kinshasa	5/10	4	*Tilapia*	≤10	1	2
7	Zaïre stream, near Kinshasa	5/10	3	*Ophiocephalus*	≤15	5	4
			1	*Clarias*	10		
			1	*Chrysichthys*	10		
			1	*Polypterus*	10		
8	Nsangi lake, 170 km south of Kinshasa	5/24	5	*Tilapia*	≤20	5	3
9	Mfidi river, 170 km south of Kinshasa	5/25	6	Sardines[a]	≤10	0	0
10	Zaïre stream, near Kinshasa	5/30	1	Sardine[a]	8	2	5
			2	Sardines[a]	≤15		
			1	*Mastacembelus*	15		
			1	Kolo-kolo[a]	15		
11	Zaïre stream, near Kinshasa	8/15	1	*Labeo*	25	3	4
			3	*Eutropius*	≥25		

[a] Scientific name unknown to the authors.
[b] From one specimen, both a mannitol-positive and a mannitol-negative biotype were isolated.

the other media, we observed no correlation between incubation temperature and the isolation of *E. tarda* or *P. shigelloides*.

Biochemical reactions of our isolates were typical for the respective species except for one mannitol-positive biotype of *E. tarda* and two *o*-nitrophenyl-β-D-galactopyranoside-negative strains of *P. shigelloides*.

Seven *E. tarda* isolates (20%) were sensitive to colistin; the remaining isolates were resistant to this antibiotic.

No *P. shigelloides* isolate agglutinated in anti-*Shigella sonnei* serum. The most common API 20E profiles for the *P. shigelloides* isolates were 7144204 and 6144204 (*o*-nitrophenyl-β-D-galactopyranoside negative). Both are rated by the manufacturer as "excellent identification."

All our isolates of *E. tarda*, with the exception of the mannitol-positive biotype, showed the same profile on API 20E: 4544000, equally rated as "excellent identification."

No other known human enteric pathogens were isolated during this investigation although the culture methods were suitable for the growth of *Salmonella* and *Arizona*. *Aeromonas hydrophila* was isolated from 14 fish.

DISCUSSION

The association of *E. tarda* with disease in humans has been reviewed by Makulu et al.

(15). The etiological relationship with diarrhea has been further substantiated by a number of recent reports from tropical countries: Madagascar (8), Tahiti (8), Panama (13), Thailand (2), VietNam (18), and Malaysia (10). In the latter two countries, *E. tarda* was considered a major enteric pathogen.

Since its description in Japan by Sakazaki in 1967 (21), *Edwardsiella* has been documented as an intestinal commensal of a wide range of reptiles and amphibians: snakes (3, 12, 13, 20, 21), crocodiles (12, 27), toads (13), frogs (23, 26), tortoises (3, 12, 20), and lizards (15, 20).

Even in tropical populations living under the most primitive hygienic conditions, it is hard to believe that direct or indirect contact with reptiles or amphibians could give rise to more than an occasional case of human infection. The existence of another reservoir host, more closely associated with humans, could therefore be postulated.

Meyer and Bullock in Arkansas (16) first mentioned the isolation of *E. tarda* from fish and considered it as a pathogen for the channel catfish. *E. tarda* was also incriminated in Japan as a cause of an epidemic outbreak in cultured crimson sea breams (14). The presence of *E. tarda* in the intestine of normal fish was never investigated before the report of Nguyen-Van-Ai (18), who isolated *E. tarda* from 44.4% of the fish examined at the Pasteur Institute of Viet-Nam in Saigon. The origin and identity of the fish were not documented. Our own work, showing an even higher isolation rate in Zaïre fish, extends this observation to another part of the tropical world and adds some preliminary information on the host species that carry this organism.

Although the human enteropathogenic potential of *P. shigelloides* has not been conclusively proved, many investigators have reported its isolation from patients suffering from diarrhea. Their work has been reviewed by Vandepitte et al. (25) and more recently by Richard et al. (19). Unlike *E. tarda*, human infections with *P. shigelloides* have also been reported from temperate countries. Moreover, besides sporadic cases, large outbreaks have been recorded. This is illustrated by the recent description by Tsukamoto et al. (24) of two epidemics of diarrheal disease in Japan attributed to the use of water contaminated with *P. shigelloides*. There are few reports on the isolation of *P. shigelloides* from surface waters (9, 24) or on the existence of potential aquatic reservoir hosts: shellfish in Tahiti (9), an oyster in Mali (25), and a xenopus toad in England (4). Infection of fish was only mentioned in France by Richard et al. (19) though it had been suggested by a case report of generalized human infection with *P. shigelloides* after puncture of the hand with a fish bone (6). Tsukamoto et al. (24) were the first to investigate healthy fish and other freshwater animals in Japan, in the search for a reservoir responsible for the human epidemics. *P. shigelloides* was cultivated from 5 out of 17 fish and also from shellfish and newts. A much higher isolation rate has not been found in Zaïrese fish and makes it highly probable that fish are a potential source for human infection with *Plesiomonas*. Evidence on the experimental enteropathogenicity of *P. shigelloides* is conflicting: although some

TABLE 2. *Isolation rates of E. tarda and P. shigelloides on different media*

Source of fish[a]	Isolation medium[b]	No. of fish examined	*E. tarda*		*P. shigelloides*	
			No. positive	Isolation rate (%)	No. positive	Isolation rate (%)
T	MC	5	5	100	3	60
R	MC	23	3	13	19	82
Total		28	8	28	22	78
T	XLD	14	10	71	4	28
R	XLD	10	2	20	6	60
Total		24	12	50	10	41
T	TB/−	19	16	84	1	5
R	TB/−	40	15	37	15	37
Total		59	31	52	16	27
T	TB/+	19	1	5	0	0

[a] T, *Tilapia* from lakes; R, river fish.

[b] Abbreviations: MC, MacConkey agar; XLD, xylose lysine desoxycholate agar; TB/−, salmonella shigella agar after enrichment for 48 h at 37°C in tetrathionate broth base without iodine solution; TB/+, salmonella shigella agar after enrichment for 48 h at 42°C in tetrathionate broth base with iodine solution.

investigators (7, 11) have detected in vitro enterotoxin production by this organism, this has not been confirmed by other workers (22, 24). We know of no studies on the experimental enterotoxic or invasive potential of *E. tarda*.

All our isolates of *E. tarda* and *P. shigelloides* were biochemically typical, except for one *E. tarda*, fermenting mannitol and sucrose, and two *o*-nitrophenyl-β-D-galactopyranoside-negative cultures of *Plesiomonas*. A mannitol-positive culture of *E. tarda* was also isolated from a case of human diarrhea in Mali (17). Among 57 isolates of *P. shigelloides* studied by Richard et al. (19), 14 were *o*-nitrophenyl-β-D-galactopyranoside negative. Most authors consider that *E. tarda* possesses a chromosomally mediated in vitro resistance to colistin (2, 17). Twenty percent of our fish isolates, however, were fully susceptible when tested with the standard disk diffusion method.

There was a significant ($P < 0.05$) difference between the isolation rates of *E. tarda* and *P. shigelloides* from river and lake fish. Of the 19 *Tilapia* fished in lakes, 16 harbored *E. tarda*, compared with 7 with *Plesiomonas*. By contrast, among 40 river fish, only 18 contained *E. tarda* compared with 28 with *P. shigelloides* (Table 1).

There were differences in isolation rates among river fish as well: the six specimens from the Mfidi river contained neither *Edwardsiella* nor *Plesiomonas*, whereas nine *E. tarda* and nine *P. shigelloides* were isolated from 11 fish of the Bombo river. A possible explanation is that the fish from the Mfidi were much smaller than the Bombo fish (Table 1). Similarly, the four small *Tilapia* of catch 6 gave only one isolate of *E. tarda*, against a 100% isolation rate among 15 adult *Tilapia* (catches 4, 5, and 8). Table 2 shows that *E. tarda* is most frequently isolated after tetrathionate enrichment without iodine solution, whereas the highest isolation rate of *P. shigelloides* is obtained after direct plating onto MacConkey agar.

E. tarda and *P. shigelloides* can be recovered at the three temperatures tested, although higher recovery rates might be expected by combining different media and incubation temperatures. Neither *Plesiomonas* nor *Edwardsiella* was found after enrichment in selenite broth, confirming a previous statement by Iveson (12).

We conclude from our observations that freshwater fish, at least some tropical species, are frequent intestinal carriers and seem to constitute the natural habitat of *E. tarda* and *P. shigelloides*. There is increasing clinical and epidemiological evidence that both bacterial species may cause diarrhea and other disease in humans. Fish should be considered as the most probable source of human infection. Further studies are needed to define the mechanism of pathogenicity of these bacteria and their ecological association with fish in different environments and geographical areas.

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